Original Article

Expression of MUC1 mucin in potentially malignant disorders, oral squamous cell carcinoma and normal oral mucosa: An immunohistochemical study

M Harish Kumar, Karpagaselvi Sanjai, Jayalakshmi Kumarswamy, Roopavathi Keshavaiah, Lokesh Papaiah, S Divya

Department of Oral Pathology and Microbiology, Vydehi Institute of Dental Sciences and Research Centre, Bengaluru, Karnataka, India

Abstract

Background: Mucins alteration in glycosylation is associated with the development and progression of malignant diseases. Therefore, mucins are used as valuable markers to distinguish normal and disease conditions. Many studies on MUC1 expression have been conducted on variety of neoplastic lesions other than head and neck region. None of the study has made an attempt to show its significance in potentially malignant disorders (PMDs) and oral squamous cell carcinoma (OSCC). Hence, ours is one of the pioneer studies done to assess and evaluate the same.

Aims: This study aims to compare and correlate the expression of MUC1 mucin protein in normal oral mucosa (NOM), PMD's and OSCC by immunohistochemical method.

Materials and Methods: Institutional study, archived tissue sections of OSCC ($n = 20$), PMD's ($n = 20$) and NOM ($n = 20$) were immunostained for MUC1 mucin and percentage of positive cells evaluated. Results obtained were statistically analyzed using Kruskal–Wallis test, Mann–Whitney test and Student's *t*-test.

Results: The mean MUC1 mucin positive cells in the study groups were as follows, 40% in OSCC, 28% in PMD's and 0.75% in NOM. Higher mean immunohistochemical score was observed in OSCC group followed by PMD's group and NOM group. The difference in immunohistochemical score among the groups was found to be statistically significant ($P < 0.001$).

Conclusion: The result of the current study suggests that determination of MUC1 mucin expression may be a parameter in the diagnosis of malignant behavior of PMD's to OSCC. MUC1 mucin expression may be a useful diagnostic marker for prediction of the invasive/metastatic potential of OSCC.

Key Words: MUC1 mucin, normal oral mucosa, oral squamous cell carcinoma, oral submucous fibrosis, potentially malignant disorders

Address for correspondence:
Dr. M Harish Kumar, Department of Oral Pathology and Microbiology, Vydehi Institute of Dental Sciences and Research Centre, #82, EPIP Area, Nalluralli Post, White Field, Bengaluru - 560 066, Karnataka, India. E-mail: mhkhrt@gmail.com

Received: 09.09.2015, **Accepted:** 20.05.2016

INTRODUCTION

Oral cancer ranks from sixth to eighth most common cancer worldwide, with a great variability in incidence among countries. In South Asia, over 90% of oral malignancies are known to arise from preexisting potentially malignant disorders (PMD's) such as leukoplakia, erythroplakia and oral submucous

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/0973-029X.185916

How to cite this article: Kumar MH, Sanjai K, Kumarswamy J, Keshavaiah R, Papaiah L, Divya S. Expression of MUC1 mucin in potentially malignant disorders, oral squamous cell carcinoma and normal oral mucosa: An immunohistochemical study. J Oral Maxillofac Pathol 2016;20:214-8.

fibrosis (OSF). Early detection of disease progression remains a challenging task mainly due to lack of adequate early prognostic markers.[1-4]

Mucins are high molecular weight glycoproteins that play a major role in cell growth, differentiation and cell signaling. Mucin gene expression is highest in the respiratory, digestive and reproductive systems.[5-9] The cancer cells use mucin for cell proliferation, survival, invasion, metastatic growth and protection against innate immunity.[6,7,10] An aberrant expression of MUC1 in various human cancers has highlighted its role in the pathogenesis of cancer.[5,7,8,11] This study was conducted to evaluate and compare the expression of MUC1 and its significance in normal oral mucosa (NOM), oral squamous cell carcinoma (OSCC) and PMD's.

MATERIALS AND METHODS

The study was conducted on the paraffin-embedded blocks retrieved from the archived files of Department of Oral Pathology and Microbiology. A total of sixty cases which were clinically and histopathologically diagnosed as OSCC ($n = 20$; well-differentiated = 13 and poorly differentiated = 7), PMD's ($n = 20$, epithelial dysplasia = 10 and OSF = 10) and NOM ($n = 20$) were stained for MUC1 mucin.

Immunohistochemical detection of MUC1 mucin

Tissues of 3.5 μm were cut and transferred to 3-amino-propyl-triethoxy-silane coated slides and incubated overnight at room temperature. Antigen retrieval of sections immersed in citrate buffer solution was done using a pressure cooker. Endogenous peroxidases were blocked (Novacastra, Leica Systems, UK) at room temperature for 15 min. Then sections were incubated with primary anti-MUC1 mucin monoclonal antibody (Thermo Scientific Pvt. Ltd., USA) for 1 h followed by incubation with biotinylated secondary antibody (Novacastra, Leica Systems, UK) for 30 min. Then a drop of streptavidin was added from secondary antibody kit (Novacastra, Leica Systems, UK) for 30 min followed by incubation with 3'diaminobenzidine-tetrahydrochloride for 5–10 min. Then the sections were counterstained with hematoxylin and mounted. Carcinoma of breast tissue [Figure 1] was used as positive control and for negative controls TRIS buffered saline replaced the primary antibody.

Interpretation of the slides

The stained sections were scanned under low power to determine the area that stained brown color and was considered as positive for MUC1 mucin expression. Cytoplasmic and membranous staining were considered as positive immunoreaction for MUC1 mucin.[12,13]

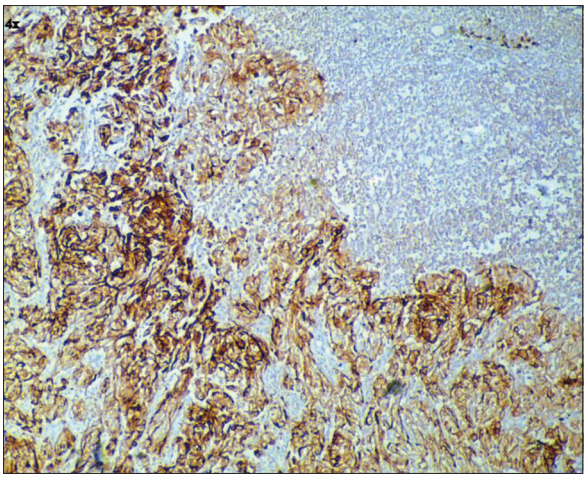


Figure 1: Photomicrograph of positive control of carcinoma of breast for MUC1 mucin (IHC stain, x40)

In a randomly selected five fields, 100 cells were considered in each field. Out of 100 cells MUC1 mucin positively stained cells were counted. Two observers evaluated all the slides.

RESULTS

In NOM, 2 out of 20 cases (0.75%) MUC1 mucin immunoreactivity was observed [Figure 2]; all the 20 cases of OSCC (44%) expressed immunoreactivity for MUC1 [Table 1 and Graph 1].

Of the twenty specimens of PMD'S, (28%) 10 of oral epithelial dysplasia exhibited membranous staining in the basal, parabasal and spinous layer cells [Figures 3 and 4]. Of 10 cases of OSF, 9 cases showed immunoreactivity in the basal, parabasal and spinous layer cells [Figure 5] and one case did not show any positivity [Table 2]. Among twenty specimens of OSCC, 13 of well-differentiated OSCC and seven of poorly differentiated OSCC showed both cytoplasmic and cell membrane staining and the distribution pattern was focal or patchy. In well-differentiated OSCC, the keratin pearls also showed immunoreactivity. Higher mean immunohistochemical score was observed in OSCC followed by PMD'S and NOM. The difference in immunohistochemical score among the groups was found to be statistically significant ($P < 0.001$).

Statistically significant difference in mean immunohistochemical score was observed between OSCC and PMD'S ($P < 0.01$), OSCC and NOM group ($P < 0.001$) as well as between PMD'S group and NOM group ($P < 0.001$). However, no significant difference in immunohistochemical score was observed between poorly differentiated OSCC and well-differentiated OSCC groups ($P < 0.301$) [Table 3 and Graph 2].

Table 1: Distribution of immunohistochemical score among the study groups

Group	Mean	SD	SEM	95% CI for mean		Minimum	Maximum	*P*
				Lower bound	Upper bound			
Oral squamous cell carcinoma	44.00	9.28	2.08	39.65	48.35	25	58	<0.001*
Potential malignant disorder	28.00	15.62	3.49	20.69	35.31	0	48	
Normal oral mucosa	0.75	2.45	0.55	−0.40	1.90	0	10	

*Significant difference. SD: Standard deviation, SEM: Standard error of mean, CI: Confidence interval

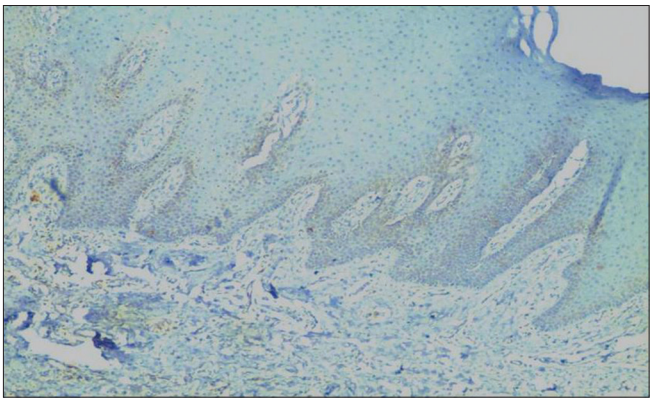


Figure 2: Photomicrograph of normal oral mucosa for MUC1 mucin (IHC stain, x100)

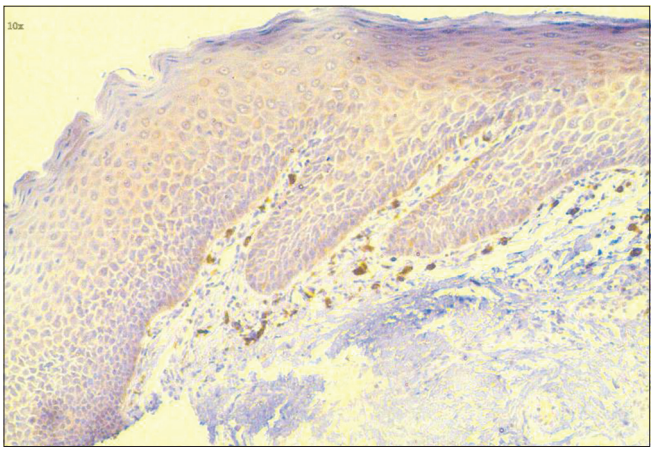


Figure 4: Photomicrograph of severe epithelial dysplasia shows faint positivity for MUC1 epithelial cells (IHC stain, x100)

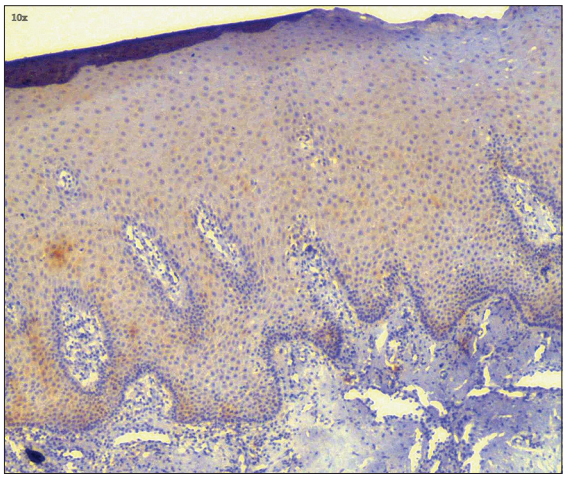


Figure 3: Photomicrograph of mild epithelial dysplasia for MUC1 shows cytoplasmic staining from basal to spinous layer of epithelium (IHC stain, x100)

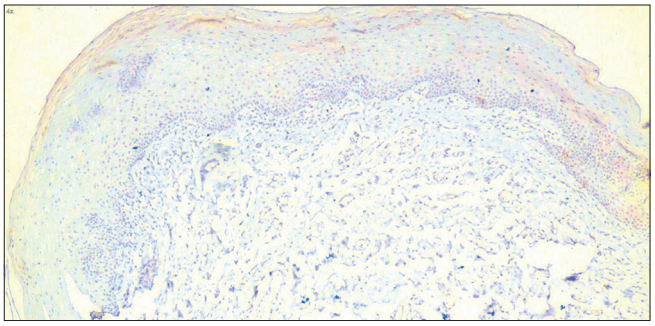


Figure 5: Photomicrograph of oral submucous fibrosis showing positivity for MUC1 in epithelial cells (IHC stain, x40)

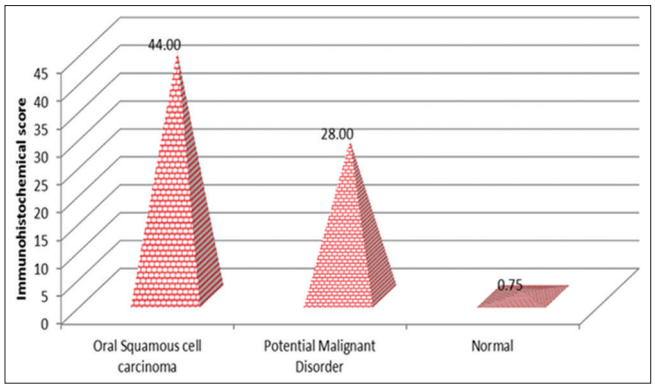
DISCUSSION

In India, OSCC is the most common cancer accounting for 12% of all cancers in men and 8% of all cancers in women.[14] In the oral cavity; OSCC is the most prevalent malignant neoplasm.

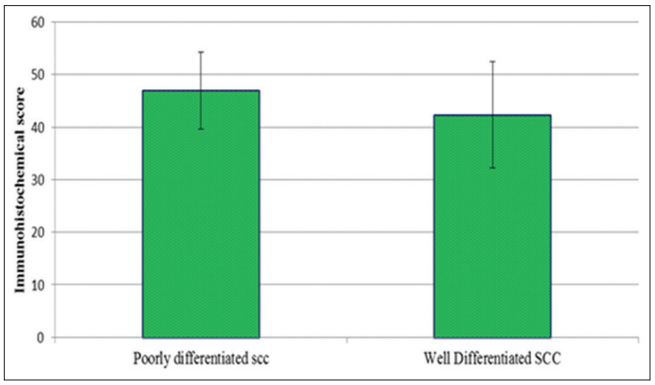
PMD's is defined by WHO 2005 as "the risk of malignancy being present in a lesion or condition either at time of initial diagnosis or a future date."[15] Leukoplakia is defined as "a white plaque of questionable risk having excluded other known diseases or disorders that carry no increased risk of cancer." Multiple studies over the years have shown a malignant transformation rate of 3.6–17.5%.[1] OSF is a chronic debilitating disease of oral cavity associated with arecanut (betel nut) chewing, affecting all parts of oral mucosa and oronasopharynx. OSF has a malignant transformation rate of about 0.5–6%.[15]

In recent years, numerous prognostic factors associated with OSCC have been identified, some of them are inherent to the patient and others associated with the genetic profile of the malignant epithelial cells which reflect tumor aggressiveness.[16]

Mucins are heavily glycosylated proteins that act as a molecular barrier and engage themselves in morphogenetic signal transduction pathways at the epithelial surface.[9,17] Mucin glycosylation content dictates the biochemical and biophysical properties of visco-elastic secretions, pointing out an important



Graph 1: Distribution of immunohistochemical score among the study groups



Graph 2: Comparison of immunohistochemical score within oral squamous cell carcinoma group

Table 2: Comparison of immunohistochemical score in potential malignant disorder group

Group	*n*	Mean	SD	SEM	Mean difference	*t*	*P*
Leukoplakia	10	40.00	6.39	2.02	24.000	5.430	<0.001*
Oralsubmucous fibrosis	10	16.00	12.43	3.93			

*Significant difference. SD: Standard deviation, SEM: Standard error of mean

Table 3: Comparison of immunohistochemical score within oral squamous cell carcinoma group

Group	*n*	Mean	SD	SEM	Mean difference	*t*	*P*
Poorly differentiated oral squamous cell carcinoma	7	47.00	7.33	2.77	4.615	1.064	0.301
Well-differentiated oral squamous cell carcinoma	13	42.38	10.08	2.80			

SD: Standard deviation, SEM: Standard error of mean

role in diverse biological functions, such as differentiation, cell adhesions, immune responses and cell signaling.[9,10,17] Their expression and alterations in glycosylation are associated with the development and progression of malignant diseases.[10,11] Therefore, mucins can be used as valuable markers to distinguish between normal and disease conditions.[5,6]

In this study, the age presentation of OSCC ranged from 27 to 76 years, with the mean age of 45.8 years. Gender distribution was 9 (45%) men and 11 (55%) women. Out of twenty OSCC cases 7 (35%) showed poorly differentiated OSCC and 13 (65%) showed well-differentiated OSCC. The age presentation of PMD's ranged from 26 to 70 years, with the mean age of 43.9 years. Gender distribution was 13 (65%) men and 7 (35%) women. Among leukoplakia cases, 2 (20%) showed mild epithelial dysplasia, 3 (30%) moderate epithelial dysplasia and 5 (50%) severe epithelial dysplasia [Table 2].

Initial studies showed that MUC1 was phosphorylated on both tyrosine and serine residues within the cytoplasmic tail and changes in phosphorylation correlates with the difference in cell adhesion.[7,9,10,17] In malignant neoplasms, aberrant glycosylation of MUC1 often leads to a reduction in the length of the carbohydrate chains and exposes normally cryptic antigens of peptide and carbohydrate nature that make MUC1 epitopes tumor-specific.[5,8,10] MUC1 mucin expression may be related to the invasion or metastasis of carcinoma cells.[12] The membrane and cytoplasm staining of MUC1 in the squamous cells might correspond to its transmembrane and cytoplasmic subunits, respectively.[10,13] A study conducted by Nitta *et al*.[12] using MUC1and Narashiman *et al*.[6] with MUC4, showed positivity in the OSCC samples which was highly restricted to the well-differentiated areas and the keratin pearls of the tumors. A similar correlation was seen in our study.

Overexpression of MUC1 in OSCC cells compared with its normal and PMD's counterpart clearly suggests role of MUC1 in the pathogenesis of OSCC, as seen in a study conducted by Nitta *et al*.[12] and Narashiman *et al*.[6] Further the cellular expression of MUC1 showed a steady increase from dysplastic noninvasive lesions to invasive OSCC.[12] Localization and identification at the ultra-structural level of MUC1 mucin in OSCC may provide important information on the role of glycoproteins in cellular malignant transformation.[11,12]

In the present study cases of mild, moderate and severe epithelial dysplasia exhibited membranous and cytoplasmic staining of MUC1 in the basal, parabasal and spinous layer cells. In most OSCC specimens, positive MUC1 mucin staining was detected both in cytoplasm and cell membranes, and the distribution pattern was focal or patchy [Figure 6] which is in accordance with Nitta *et al*. in 2000.[12] The immunoreactivity of OSCC also depended on the degree of cellular differentiation (keratinization) as seen in Nitta *et al*.[12] and Narashiman *et al*.[6] Peripheral cells of epithelial islands were stained intensely with a decrease in immunoreactivity toward the center of such islands.

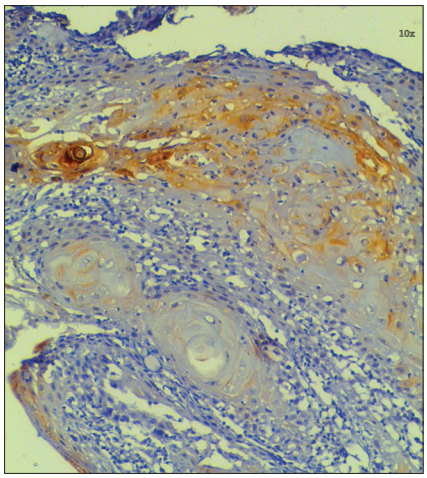


Figure 6: Photomicrograph of the section shows MUC1 of keratin pearl in well-differentiated squamous cell carcinoma and patchy staining pattern in other squamous cells (IHC stain, x40)

In this study, statistically significant difference in mean immunohistochemical score was observed between OSCC and PMD's group ($P < 0.01$), OSCC and NOM group ($P < 0.001$) as well as between PMD's group and NOM group ($P < 0.001$) [Table 1 and Graph 1].

Nitta *et al*. in their study showed statistically significant difference between NOM and epithelial dysplasia ($P < 0.01$), between NOM and carcinoma *in situ* ($P < 0.01$), between NOM and OSCC ($P < 0.01$), and between epithelial dysplasia and OSCC ($P < 0.01$). Dominant cytoplasmic expression was found be increasing from premalignant to malignant lesions ($P < 0.001$).[12]

CONCLUSION

The present study infers up-regulation of MUC1 mucin expression in PMD's and malignant lesions might play a vital role in the pathogenesis and its progression. It can also be a useful diagnostic marker for prediction of the invasive/metastatic potential of OSCC. Hence, MUC1 mucin can be regarded as a useful marker for PMD's and OSCC.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Marocchio LS, Lima J, Sperandio FF, Corrêa L, de Sousa SO. Oral squamous cell carcinoma: An analysis of 1,564 cases showing advances in early detection. J Oral Sci 2010;52:267-73.
2. Markopoulos AK. Current aspects on oral squamous cell carcinoma. Open Dent J 2012;6:126-30.
3. Neville BW, Day TA. Oral cancer and precancerous lesions. CA Cancer J Clin 2002;52:195-215.
4. Warnakulasuriya S, Johnson NW, van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. J Oral Pathol Med 2007;36:575-80.
5. Mahomed F. Recent advances in mucin immunohistochemistry in salivary gland tumors and head and neck squamous cell carcinoma. Oral Oncol 2011;47:797-803.
6. Narashiman S, Narasimhan M, Venkatraman G. Expression of mucin 4 in leukoplakia and oral squamous cell carcinoma: An immunohistochemical study. J Oral Maxillofac Pathol 2014;18:25-31.
7. Hollingsworth MA, Swanson BJ. Mucins in cancer: Protection and control of the cell surface. Nat Rev Cancer 2004;4:45-60.
8. Rachagani S, Torres MP, Moniaux N, Batra SK. Current status of mucins in the diagnosis and therapy of cancer. Biofactors 2009;35:509-27.
9. Croce MV, Price MR, Segal-Eiras A. Detection and isolation of MUC1 mucin from larynx squamous cell carcinoma. Pathol Oncol Res 2000;6:93-9.
10. Pereira MC, Oliveira DT, Landman G, Kowalski LP. Histologic subtypes of oral squamous cell carcinoma: Prognostic relevance. J Can Dent Assoc 2007;73:339-44.
11. Yonezawa S, Sato E. Expression of mucin antigens in human cancers and its relationship with malignancy potential. Pathol Int 1997;47:813-30
12. Nitta T, Sugihara K, Tsuyama S, Murata F. Immunohistochemical study of MUC1 mucin in premalignant oral lesions and oral squamous cell carcinoma: Association with disease progression, mode of invasion, and lymph node metastasis. Cancer 2000;88:245-54.
13. Itoh T, Yonezawa S, Nomoto M, Ueno K, Kim YS, Sato E. Expression of mucin antigens and lewis X-related antigens in carcinomas and dysplasia of the pharynx and larynx. Pathol Int 1996;46:646-55.
14. Raghu R, Bijayata S, Dipshikha B. Oral cancer- an overview. In: Kalu U. E. Ogbureke, editor. Oral Cancer. 1st ed. Croatia: InTech publications; 2012. Avaliable from: http://www.intechopen.com/books/oral-cancer/oral-cancer-an-overview. [Last accessed on 2016 Jun 28].
15. George A, Sreenivasan BS, Sunil S, Varghese SS, Thomas J, Gopakumar D, *et al*. Potentially malignant disorders of oral cavity. J Oral Maxillofac Pathol 2011;2:95-100.
16. Rabassa ME, Croce MV, Pereyra A, Segal-Eiras A. MUC1 expression and anti-MUC1 serum immune response in head and neck squamous cell carcinoma (HNSCC): A multivariate analysis. BMC Cancer 2006;6:253.
17. Croce MV, Rabassa ME, Pereyra A, Segal-Eiras A. Differential expression of MUC1 and carbohydrate antigens in primary and secondary head and neck squamous cell carcinoma. Head Neck 2008;30:647-57.